REMARKS

Status of the Claims

Claims 1 to 94 have been cancelled. Claims 95 to 97 have been amended. Claims 95-109 are pending and under consideration.

Claim 95 and 102-109 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The rejection of Claims 95 and 102-109

Claims 95-109 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

According to the Office Action:

> Claim 95 is vague and indefinite because it lacks active methods steps by which to carry out the "immunoglobulin steroid hormone inhibition assay" and the "immunoglobulin steroid inhibition positive control assay". As the claim is now drafter a sample is simply treated to remove steroid hormones and added to steroid hormone receptor responsive tumor cells; the control sample is the addition of IgA or IgM to steroid hormone responsive cells. the claim requires that the "concentration" be determined at which said treated sample inhibits steroid hormone mediated cell growth. however, since the treated sample contains no steroid hormone and the cell lines are steroid hormone responsive, it would be expected that no growth would result due to lack of steroid hormones, irrespective of the presence or absence of an immunoglobulin steroid hormone response inhibitors in the sample. Further, it is unclear how a "concentration" is to be determined without knowing what the concentration is of Concentration is normally given in mass per unit volume, but the claim does not specify the molecule which is to be measured by concentration. The treated sample is any sample wherein steroid hormones have been effectively removed. When given the broadest reasonable interpretation, the treated sample can comprise a cell

lysate. There are many molecules within the cell lysate which can be represented as mass per unit volume, and without a specific active method step dictating how the concentration is to be calculated, the metes and bounds of the claimed method cannot be determined.

A steroid hormone is not required for cell growth

With regard to the Examiner's belief that steroid hormone is required to allow cell growth, Applicant would like to point out that added steroid hormone is simply not required for cell growth according to Applicant's disclosure. This is demonstrated throughout the present application. Please see Figures 72-77. As shown in many of these Figures, when there is no added inhibitor, the cell population doublings are the same whether estrogen was added or not. As illustrated in the cited Figures, estrogen only had an effect on growth when an inhibitor was present, whereupon the estrogen overcame the inhibitory action and promoted cell growth.

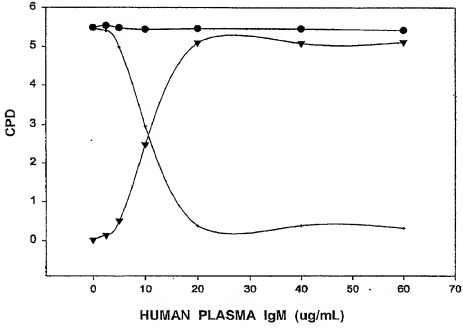
Figure 72 (see below) illustrates the effect of IgM isolated from human plasma on MTW9/PL2 growth under serum-free conditions. The solid circles indicate cell growth when estrogen is added, the small dots indicate cell growth with no estrogen added. At a Human Plasma IgM level of 0 (no added inhibitor), the cell growth is essentially identical for both cultures where there is added estrogen and where no estrogen is added, with a cell population doubling of about 5.5. Other Figures cited provide similar results. The data illustrated in Applicant's Figures demonstrates that cell growth will occur without added estrogen. The cells are estrogen responsive, but that does not mean estrogen is required for cell growth.

The Concentration Term is clarified in Amended Claim 95

Claim 95 has been amended to remove the concentration term, and add reference to the amount of specific material added to the cultures, thus defining explicitly the comparison that is to be carried out, that is comparing the amount of added plasma immunoglobulin to the amount of treated sample added. This amendment to the claims that allows for the comparison of specific amounts of added materials allows the metes and bounds of the claim to be determined.

FIGURE 72

EFFECT OF IgM ISOLATED FROM HUMAN PLASMA
ON MTW9/PL2 GROWTH IN SERUM-FREE CONDITIONS



LEGEND:

—●— = + E_2

—— = − E_2

—▼— = Estrogenic effect

Amended Claim 95 includes "Active Steps"

Each element of Claim 95 is an active step. From the data presented in the specification at the indicated points, the steps of conducting an immunoglobulin steroid hormone inhibition assay of steroid-hormone responsive tumor cells in serum-free media would be an active step, as cell growth is expected for at least the positive control. Treating a sample and determining amounts are specific active steps. The method is fully defined and supported in the specification. In view of the amendments and comments provided above we believe Amended claim 95 is definite and that it complies with the

requirements of 35 U.S.C. §112. We respectfully ask that the rejection of claim 95, as well as dependent claims 102-109 be withdrawn. Claims 102 to 109 are dependent on claim 95 and detail specific cell lines or groups of cell lines that have been enumerated in the Markush group in claim 95.

The rejection of Claims 95-109

According to the Office Action:

> Claims 95 and 107 are drawn in part to a method requiring the steroid-hormone responsive cell line of GH4C1. Claim 108 requires the cell line of GH4C1. The originally filed disclosure fails to provide a description of GH4C1 cells. thus, one of skill in the art would reasonably conclude that applicant was not in possession of the claimed methods requiring said cells.

Cell Line GH4Cl is fully disclosed

Applicant's original specification includes several references to the named cell line, GH4C1. Some of these references include Table 1 after paragraph [0214] of the application as submitted (or paragraph [0228] of the published application 2002/0006630), and Figures 87 to 89. The reference given in Table 1 (Tashjian AH Jr (1979) *Methods Enzymol.* **58**, 527-535, ER^+ rat pituitary tumor) indicates that this cell line is known in the art and described in literature since at least 1979, far before the filing date of the present application. As evidenced by the multiple uses given in the specification as filed and reference to literature predating the application, this cell line was in possession of the inventor at the time the application was filed and utilized to generate data described in the Examples and provided in the Figures. In view of Applicant's disclosure of the cell line, GH4Cl, as noted, Applicant respectfully requests that this rejection be withdrawn.

The Office Action further states:

> Claims 96 and 97 are drawn to methods wherein a samples of mucosal epithelial cultured cells is treated with polymeric IgM or polymeric IgA and another identical sample is left untreated. Bothe the treated and untreated cell samples are incubated under growth promoting conditions; and the cell population doublings of the cell samples are measured after the incubations, wherein a lack of increase in the cell population doublings of the cell sample treated with polymeric IgM or

polymeric IgA with respect to the untreated samples is indicative of the presence of the mediator of immunoglobulin inhibition of steroid hormone responsive cell growth.

The originally filed disclosure fails to support the newly added claim. The specification states only that a method of detecting a mediator of immunoglobulin inhibition of steroid hormone responsive cell growth includes the detection of a poly-Ig receptor in a mucosal epithelial cell (para [0026]).

Advisory Action states:

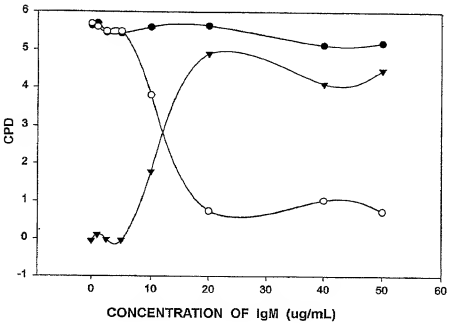
Applicant has submitted an amendments of claims 96 and 97 to specify that cell growth is inhibited by polymeric IgM rather than a mediator of immunoglobulin inhibition of steroid hormone responsive cell growth. Applicant argues that reference to the detection of a "mediator of immunoglobulin inhibition of steroid hormone responsive cell growth has been deleted. This is not found persuasive as claims 96 and 97 are still drawn to a method of detecting a mediator of immunoglobulin inhibition of steroid hormone responsive cell growth as the method objective.

Applicant's Specification supports Amended Claims

The relevant claims have been amended to remove all the reference to detecting a mediator of immunoglobulin inhibition in the elements of the claims as well as the preamble. The amended comparing step is used to determine whether the cells are inhibited or not by polymeric IgM or plasma IgA, which is described in paragraphs [0379] and [0385] of the application as submitted (or paragraphs [0537] and [0549] of the published application 2002/0006630). These paragraphs describe the use of IgA and IgM as inhibitors of cell growth in steroid hormone responsive cells. Utilization of these inhibitors according to the claimed method enables the testing of other cells for the same type of response, i.e. cell growth inhibition. The data provided in FIG. 62 (see below) displays a typical response between a cell sample untreated with IgM (0 μg/ml of IgM) and a cell sample treated with IgM (>20 μg/mL of IgM). The newly amended claims are now fully supported in the specification, and we respectfully request that the rejection of claims 96 and 97 be withdrawn.

FIGURE 62

EFFECT OF HORSE IgM ON GROWTH OF THE MTW9/PL2 CELLS IN 2.5% CDE HORSE SERUM $\pm E_2$



LEGEND:

= + E_2

= − E_2

= Estrogenic effect

From the application as filed:

Paragraph [0379] **Fig. 62** shows very clearly that commercially prepared horse serum derived IgM (Custom Monoclonals), was very active. At concentrations of 20 to 50 μg/mL, IgM completely inhibited the growth of the MTW9/PL2 cells (i.e. < 1.0 CPD). Addition of 10 nM E_2 reversed the inhibition nearly completely. Estrogenic effects of 4 to 5 CPD were seen (**Fig. 62**). **Fig. 63** shows the same general results with commercially prepared horse serum derived IgA (Accurate). The only apparent difference was that IgA was slightly more effective than IgM.

These results clearly proved that the active components in CA-PS-pool II were IgA and IgM.

Paragraph [0385] Rat IgA was a potent estrogen reversible inhibitor (**Fig. 68**). At 20 to 50 μg/mL, it completely inhibited growth. Addition of 10 nM E2 completely reversed the inhibition. The estrogenic effects recorded were > 5 CPD. The results with rat IgM were very similar (**Fig. 69**). At 20 to 50 μg/mL, it completely inhibited growth. Addition of 10 nM E 2 reversed the inhibition. The estrogenic effects recorded were > 5 CPD. It is essential to note that IgA or IgM replaced the effect of full CDE-rat serum with MTW9/PL2 cells. With a completely homologous system (i.e. cell line, basal 2.5% CDE-serum, and immunoglobulins), the results were clear. IgA and IgM were the sought after serum-borne inhibitors from rat.

Control Sample is disclosed

Advisory Action further states:

> Applicant argues that a person of ordinary skill in the art would clearly recognize that significance of a comparison of the results of a negative control sample containing the substance to be assessed and also be capable of carrying out such a comparison without undue experimentation. Applicant is reminded that the rejections were not for lack of enablement based on undue experimentation, but lack of written description. Applicant is reminded that disclosure in an application that merely renders the later-claimed (by amendment) invention obvious is not sufficient to meet the written description requirement of 35 USC 112, first paragraph.

Example 7 of the present application describes testing Phenol Red for estrogenic activity. In the discussion of example 7, paragraph [0274] of the application as filed (paragraphs [0339] and [0340] of the published application 2002/0006630) states

> The studies of the effects of phenol red or its lipophilic impurities demonstrate the usefulness of the presently disclosed methods for the assessment of estrogenic and androgenic activity of commercially prepared materials, substances present or extracted from the environment or food sources that are thought to contain such activities.

The test methods described in example 7 compare cell growth in samples with and without phenol red present (the substance of interest). The sample without phenol red are negative control tests. These testing methods are described in paragraphs [0263], [0266], [0268], and [0271] of the application as filed to provide a number of examples on

"control tests" (paragraphs [0319], [0324], [0328], [0333] and [0334] of the published application 2002/0006630).

From the application as filed:

Paragraph [0263] As shown in Fig. 20A, growth was measured in the presence of increasing concentrations of CDE-horse serum with and without phenol red in the medium and ± E_2.

Paragraph [0266] T47D cells were grown in medium with CDE-horse serum both with and without phenol red (**Fig. 21A**). Low concentrations of serum (i.e. < 2%) promoted growth. Higher concentrations progressively inhibited growth irrespective of indicator content. In both media, E2 was required to reverse the inhibition (**Fig. 21A**). In 50% (v/v) CDE-horse serum, the maximum E2 responses were $2^{5.35}$ (41-fold) and $2^{5.29}$ (39-fold) in phenol red containing and indicator free medium, respectively (**Fig. 21B**).

Paragraph [0268] The maximum estrogenic effects in 50% serum were $2^{5.82}$ (56-fold) and $2^{5.69}$ (52-fold) with and without phenol red, respectively (**Fig. 22B**). In the experiment shown in **Fig. 22B**, estrogenic effects were unpredictably greater in phenol red free medium than in medium with indicator.

Paragraph [0271] **Comparison of E2 Potency in Medium with and without Phenol Red**. As described above in **Table 4**, the T47D and MTW9/PL2 cells grow significantly in response to 1.0×10^{-12} M E_2. The D-MEM/F-12 used in those studies also contained about 23μM phenol red. When the results of those studies were compared to the experiments in **Fig. 23B**, done in D-MEM/F-12 without indicator, the estrogen dose response curves were very similar. The conclusion is straightforward. E_2 dose-responses were not affected by phenol red.

As shown in each of the excerpts from the application, a sample is tested with and without phenol red, the substance of interest in these examples, and a comparison is carried out between the samples to determine the effect phenol red has as an estrogenic compound. The discussion of example 7, stated above, demonstrates that the inventor contemplated the use of these same tests with other substances of interest in general. The testing of other substances following the examples with Phenol Red would be carried out using medium with and without the substance present, to determine whether such substances possess any estrogenic activity. Samples without any added material of

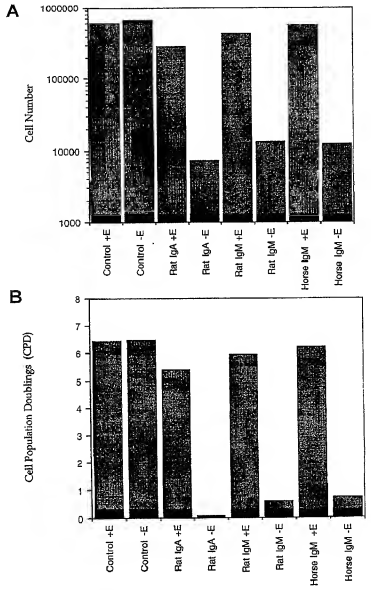
interest would be immediately recognized by one skilled in the art as control samples, which are needed to be able to assess the affect of the substance of interest on the sample.

Also, figures in the filed application show that the applicant clearly disclosed a control as a sample without a material present because many figures compare cell growth with and without immunoglobulin inhibitor present (FIGS 62, 63, 72, 74, 80, 82, 85, 87, 93-96, 98, 99, 101, 102 and 104 at least). Especially Figure 73 (see below), which displays a bar graph of the effects of various types of IgA or IgM with and without estrogen present on cell growth. The graph in Figure 73 labels the Cell Number or Cell Population Doubling without IgA or IgM present (furthest to the left) as a control.

A person of ordinary skill in the art would clearly recognize the significance of a comparison of the results of a control to a test sample containing the substance to be assessed and also be capable of carrying out such a comparison. This type of testing, utilizing a control sample, is expected to be done when assessing affects of a material on a cell culture whether or not the control sample was explicitly described in a procedure (see paragraph 4 of the inventor's declaration). The control sample provides a baseline to compare against another sample that contains the substance of interest, and this type of comparison is routinely carried out in the biochemical art and would be inherent in a comparative test (see paragraph 6 of the inventor's declaration). Also, an explicit description of such a control sample, where a material is not added to a cell culture, is not needed, because it is well recognized in the biochemical art that such a control sample will be used, and describing the preparation and use of the sample is implied when carrying out such an assessment (see paragraph 5 of the inventor's declaration). Controls are added to each type of assay to identify possible alternate reasons for either negative or positive results, and these are common knowledge facts that do not need reiteration in experimental procedures (see paragraph 7 of the inventor's declaration). The meaning of significant increases in cell population doubling is supported in paragraphs [0221] and [0222] of the application as filed (or paragraphs [240] and [0241] of the published application 2002/0006630).

FIGURE 73

THE EFFECT OF VARIOUS IgA AND IgM PREPARATIO
ON MTW9/PL2 CELLS GROWN IN SERUM-FREE MEDI



The Office Action further states:

Claims 98-101 are drawn to methods of detecting estrogenic activity of a substance of interest comprising adding an inhibitory amount of IgM or IgA to at least two or thee samples of a maintained steroid hormone responsive cancer cell population; adding an amount of the

substance of interest to one of the cell samples to yield a test mixture; designating the samples without any substance as a control; incubating the samples for a period of time; measuring the cell population in the samples after the period of time and comparing the test mixture cell population doublings with the control mixture cell population doubling, wherein a significant increase in cell population doublings in the test mixture indicates that the substance of interest possesses estrogenic activity.

the originally filed disclosure states that the estrogenic effect is calculated as the difference between cell population doublings in the presence and absence of steroid, which does not provide for the cell samples with and without IgA or IgM. One of skill in the art would reasonably conclude that applicant was not in possession of the claimed methods at the time of filing.

No samples required in claims 98 to 101 are used without the addition of IgA or IgM

Applicant respectfully draws the Examiner's attention to the specifics of claims 98-101, introduced in response to the previous Office Action. Each of these claims require the addition of either IgA or IgM to all samples that are utilized in the claims. For example, claim 98 states in part:

> adding an inhibitory amount of IgA to at least two samples of maintained steroid hormone-responsive cancer cell population in a nutrient medium;

No samples required in any of claims 98 to 101 are used without the addition of IgA or IgM.

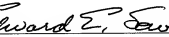
Applicant was in possession of Claimed Invention at the time of Filing

Applicant's claimed method of testing (claims 98-101) is described in Applicant's originally filed specification, and utilizes the estrogenic effect as a very sensitive determiner of estrogenic activity of a substance. Applicant's Example 18 describes the estrogenic effect observed for various cell lines and discusses its usefulness as a way of assessing estrogenic activity. More specifically, paragraph [0405] of the application as filed (or paragraphs [0579] and [0580] of the published application 2002/0006630) provide:

> [0405] **Discussion of Example 18**. These methods will permit evaluation of industrial, environmental, biological, medical, veterinary medicine and other potential sources of estrogenic or androgenic activity under the most sensitive conditions yet developed. Estrogenic activity is measurable at <1.0 picomolar concentrations.

Because the pending claims, when properly understood, particularly point out and distinctly claim the subject matter Applicant regards as his invention, because the pending claims are based on an adequate written description, and the pending claims are fully supported by Applicant's specification as originally filed, Applicant respectfully requests that the current rejections be withdrawn and that claims 95-109 be allowed.

Respectfully submitted,

By 

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